Role of polyunsaturated fatty acids and lipid peroxidation on colorectal cancer risk and treatments

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Abstract
The review aims at elucidating the role of lipid peroxidation of polyunsaturated fatty acids (PUFAs) in colorectal cancer (CRC) risk and treatment.

Reference

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Role of polyunsaturated fatty acids and lipid peroxidation on colorectal cancer risk and treatments

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Purpose of review
The review aims at elucidating the role of lipid peroxidation of polyunsaturated fatty acids (PUFAs) in colorectal cancer (CRC) risk and treatment.

Recent findings
CRC is one of the most overriding threats to public health. Despite a broad range of treatments, up to 50% of patients will inevitably develop incurable metastatic disease. Peroxidation of PUFAs contributes to augmentation of oxidative stress and causes in consequence inflammation, which is one of the possible carcinogenic factors of CRC. End products of PUFAs might be used as biomarkers for CRC detection and surveillance for treatment. They also have cytotoxic effect in CRC cells. Experimental results suggest that ω-3 PUFAs could increase the efficacy of radiotherapy and chemotherapy of CRC.

Summary
Lipid peroxidation, one factor of oxidative stress, might play a paramount role not only in carcinogenesis but also in potential therapeutic strategy on CRC. End products of lipid peroxidation, such as malondialdehyde, 4-hydroxy-2-nonenal, and isoprostanes, could be used as biomarkers for cancer detection, surveillance of treatment outcome and prognostic index for CRC patients. Furthermore, malondialdehyde and 4-hydroxy-2-nonenal have cytotoxic effect not only in normal cells but also in CRC cancer cells, which implies the potential role of PUFAs in CRC treatment.

Keywords
colorectal cancer, lipid peroxidation, oxidative stress, polyunsaturated fatty acids

INTRODUCTION
Colorectal cancer (CRC) incidence is increasing globally, whereas current treatments do not sufficiently reduce cancer recurrence and mortality. New therapeutic modalities are thus under investigation. Among them, nutritional supplementation could increase effectiveness of conventional treatments and reduce side-effects on healthy tissues. Recent studies suggest that ω-3 polyunsaturated fatty acids (PUFAs) can enhance oxidative stress in cancer cells and decrease inflammatory response in normal tissues during radiotherapy and chemotherapy.

Epidemiology and cause
Colorectal cancer is estimated as the third leading cause of cancer mortality in the USA in 2010 [1]. In the USA, CRC incidence and mortality are negatively associated with socioeconomic status, which can be explained by the difference of several CRC-related factors, such as diet and obesity [2]. Socioeconomic status and comorbidities are the most important factors for disease-free survival of CRC [3]. As suggested by these observations, socioeconomic and cultural factors, such as nutritional status (e.g. poor nutrition and obesity), education, compliance to treatment, post-treatment surveillance and health insurance, are important in CRC burden and survival.

More particularly, CRC pathogenesis is closely related to lifestyle, especially the diet composition. ‘Western diet’ defined by high fat, high protein, high energy intake, but low fiber, low calcium and low vitamin D consumption is the most threatening diet composition [4]. Immigrants from...
low-risk to high-risk countries approach the incidence of their adopted countries within one to two generations, emphasizing the paramount influence of environmental factors on CRC cause [5]. Because population ageing and Western lifestyle becomes widespread, CRC emerges as an overriding threat to life expectancy and quality.

**TREATMENT MODALITIES**

Colorectal cancer treatment schema (Fig. 1) [6,7] is programmed according to the patients’ age, medical history, health status, and tolerance for specific medications. Depending on tumor stage and localization, it consists of colectomy or laparoscopic surgery followed by adjuvant or palliative radiotherapy or chemotherapy. Even though, 50% of all newly diagnosed patients will ultimately develop incurable metastatic disease, regardless of treatment, with a median overall survival of about only 20 months [8]. Furthermore, side-effects, such as nausea, vomiting and weight loss, induced by the conventional treatments are frequent; therefore new therapeutic modalities that can improve the

**FIGURE 1.** Treatment modalities of colorectal cancer. Colorectal cancer treatment is chosen according to the TNM system [6]. T, tumor; N, lymph node; M, metastasis. T1: the cancer has grown through the muscularis mucosa and extends into the submucosa; T2: the cancer has grown through the submucosa and extends into the muscularis propria; T3: the cancer has grown through the muscularis propria and into the outermost layers of the colon or rectum but not through them; T4: the cancer has grown through the serosa or reached any nearby organs or tissues. N0: no cancer in nearby lymph nodes; N1: cancer cells are found in 1 to 3 nearby lymph nodes or small deposits of cancer cells are found in areas of fat near lymph nodes, but not in the lymph nodes themselves; N2: cancer cells are found in more than four nearby lymph nodes. M0: no distant spread is seen; M1: the cancer has spread to distant organs or set of distant lymph nodes [6]. High-risk factors of stage II disease comprise age, poor differentiated tumor, perforation/occlusion, T4, and examined lymph nodes below 12. Recently, additional biological targeted treatments, such as angiogenesis inhibitors and epidermal growth factor receptor inhibitors, are used to increase the effect of conventional treatments [7].

**KEY POINTS**

- End products of PUFAs, MDA, 4-hydroxy-2-nonenal (4-HNE) and isoprostanes, might be used as biomarkers for detection of colorectal cancer, surveillance of treatment outcome and prognostic index.
- Both MDA and 4-HNE have a double-edged sword effect in normal or cancer cells.
- End products of PUFAs might also have therapeutic potential in colorectal cancer.

![Diagram of Colorectal Cancer Diagnosis and Treatment Modalities](image-url)
efficacy of conventional treatments while reducing their adverse effects are welcome.

OXIDATIVE STRESS AND INFLAMMATION IN CARCINOGENESIS

Chronic oxidative stress can cause a continuous local inflammatory response that would induce tissue destruction and regeneration, so-called chronic inflammation. Chronic inflammation is considered as a preneoplastic state of CRC, by inducing gene mutations, inhibiting apoptosis, or stimulating angiogenesis and cell proliferation [9]. One of the inflammation-related mediators, nuclear factor kappaB (NF-κB), represents a family of transcriptive factors, which regulate the expression of genes. Many of these genes are involved in inflammation and apoptosis. Constitutively active NF-κB could promote tumor growth and was found in 67% of CRC cell lines and in 40% of CRC tissues [10]. Apart from this, many human cancers exhibit elevated prostaglandin levels due to up-regulation of cyclooxygenase-2 (COX-2), an enzyme responsible for some important inflammatory mediators. COX-2 is also significantly overexpressed in CRC [11]. Talero et al. [12*] characterized chronic inflammation and CRC-associated biomarker expression, such as β-catenin, COX-2, and inducible nitrogen oxide synthase as well as proinflammatory cytokines such as tumor necrosis factor (TNF)-α, interferon (IFN)-γ, and IL-6, were highly associated with transformation from colitis to CRC. Long-term ingestion of antioxidants could reduce CRC incidence in rats by deoxygenating oxidative stress. In the same animal model, the amount of PGE2 and COX-2 in CRC were significantly elevated compared with that in the normal mucosa. In aberrant foci crypts, production of PGE2 and expression of COX-2 were inhibited by the antioxidant [13]. These observations suggest that oxidative stress and inflammation play an important role in CRC carcinogenesis, and that decreased oxidative stress would therefore influence CRC risk.

ROLE OF POLYUNSATURATED FATTY ACIDS

Dietary intake of ω-3 PUFAs is reported to be associated with decreased risk of CRC [14], which is possibly due to decrease of oxidative and inflammatory markers; whereas a high ω-6/ω-3 PUFA ratio is associated with increase of inflammation [15]. The fact indicates that PUFAs might influence CRC risk by regulating systemic inflammation status.

In preneoplastic lesions of CRC, ω-3 PUFA-enriched diet supplementation is beneficial in a rat model of colitis via inhibition of inflammation and oxidative stress [16]. ω-3 PUFAs suppress colonic polyps in a mouse model, which is possibly due to a decreased level of ω-6 PUFAs in colic mucosa [17*]. Thereafter this decrease causes a reduction in COX-2 expression and β-catenin nuclear translocation. These molecular changes result in inhibition of polyp hyperplasia [17*]. These indicate that ω-3 PUFAs could reduce inflammation in colitis and colonic polyps, which consequently decrease risk transformation to CRC.

ω-3 PUFAs could not only play a preventive role against CRC, but also be involved in CRC treatment, displayed as a reduction of cell growth in a time and dose-dependent manner both in vitro and in vivo with or without radiotherapy or chemotherapy [18,19]. ω-3 PUFA supplementation selectively increased its concentration in the colonic mucosa and leads to a reduction in cell proliferation and an increase in apoptosis in the crypts in patients with a history of CRC [20]. ω-3 PUFAs can thus be used as a targeted treatment combined with radiotherapy or chemotherapy for CRC.

Different from ω-3 PUFAs, ω-6 PUFAs have been described to stimulate CRC cell growth [21]. A large population-based prospective study shows that ω-3 PUFA intake is negatively correlated with the risk of CRC only in proximal colon but not in distal colon and rectum. But neither ω-6 PUFA increases CRC risk, nor elevated ω-3/ω-6 ratio decreases CRC risk [22**]. Lu et al. [24*] found that ω-6 PUFAs could promote cell proliferation when used in a low concentration (100–200 μmol/l) in both well differentiated and undifferentiated CRC cell lines; when used in a higher dose (300 μmol/l), ω-6 PUFAs had a suppressive effect in CRC cells [23] by altering cell membrane composition, and this alteration depends on cell differentiation status [24*]. Dietary supplementation with ω-3 PUFAs might thus play an important role in CRC chemoradio-prevention, whereas the role of ω-6 PUFAs remains currently inconclusive.

METABOLIC PATHWAYS OF POLYUNSATURATED FATTY ACIDS

After being absorbed into cells either by simple diffusion or via specific fatty acid transporter proteins in the membrane, nonesterified fatty acids are converted to fatty acid acyl-CoA thioesters [25]. Absorbed fatty acid acyl-coenzyme A can be transformed into long-chain PUFA through an elongation or desaturation process, in which ω-3 PUFAs and ω-6 PUFAs are competitively metabolized [25].

The metabolites of PUFAs (Table 1) actively participate in cell signal transduction pathways,
and to some degree, regulate gene expression. Generally, ω-3 PUFAs produce 3-series prostaglandins and 5-series leukotrienes by cyclooxygenase-1 (COX-1), whereas the ω-6 PUFAs give rise to 2-series prostaglandins and thromboxanes and 4-series leukotrienes by COX-2 [26]. Increased consumption of ω-3 PUFAs could consequently inhibit ω-6 PUFA-derived prostaglandin synthesis from COX-2. Prostaglandin E₂, one of the products of ω-6 PUFAs via COX-2 pathway, has been involved in CRC carcinogenesis [21]. Therefore inhibition of inflammatory responses, such as COX-2 induction and prostaglandin E₂ synthesis, may be effectively involved in the antitumor effect of ω-3 PUFAs and in the difference between ω-3 and ω-6 PUFAs mentioned above.

**Table 1. Metabolic products of ω-3 and ω-6 polyunsaturated fatty acids**

<table>
<thead>
<tr>
<th>PUFAs</th>
<th>Sources</th>
<th>Metabolic products</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>ω-3 PUFAs</td>
<td>Docosahexanoic acid</td>
<td>Prostanoids (PGE₂, PGE₃, PGI₂, TXA₃)</td>
<td>Vasodilatation inflammation inhibition</td>
</tr>
<tr>
<td></td>
<td>Eicosapentaenoic acid</td>
<td>Leukotrienes (LTB₄, LTC₄, LTD₄)</td>
<td>Vasoconstriction inflammation induction platelet aggregation and adhesion branchoconstriction</td>
</tr>
<tr>
<td>ω-6 PUFAs</td>
<td>Linoleic acid</td>
<td>Prostanoids (PGE₂, PGE₃, PGF₂, PGF₃, PGA₁, TXA₃)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Leukotrienes (LTB₄, LTC₄, LTD₄)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lipoxins (LXA₄, LXB₄, LXC₄, LXD₄, LTE₄)</td>
<td></td>
</tr>
</tbody>
</table>

**LIPID PEROXIDATION OF POLYUNSATURATED FATTY ACIDS**

Apart from prostaglandins, lipid peroxidation products are the most investigated consequences of PUFAs metabolism. Lipid peroxidation not only provokes cell membrane disruption but also generates a complex range of reactive carbonyl compounds, ketones and alkanes (Fig. 2). These compounds will finally produce a series of lipid peroxidation end products. Among them, malondialdehyde (MDA) is the most mutagenic, whereas 4-hydroxy-2-nonenal (4-HNE) is the most toxic [27]. Isoprostanes (IsoPs) are a series of prostaglandin-like compounds produced by the free radical-catalyzed peroxidation independent of COX pathway (Fig. 3) [28]. These end products of lipid peroxidation participate in the signal transduction cascade, cell proliferation and differentiation control, as well as in apoptosis pathways.

**Malondialdehyde, the most mutagenic product of lipid peroxidation**

Malondialdehyde is one of the end products of peroxidation of PUFAs. Dietary intake of PUFAs increases intestine MDA production *in vivo* [29]. It then reacts with nucleic acid bases to form adducts such as the pyrimido[1,2a]purin-10(3H)-one (M₄dG). If the DNA repair system excises and replaces the M₄dG with a normal DNA base, cells will undergo normal cell cycle; if not, either the cells will undergo apoptosis or genetic mutation will be induced. The genetic mutation caused by M₄dG is one of the possible reasons for the carcinogenic feature of MDA. MDA together with 4-HNE also form adducts with elongation factor two in ribosome, that consequently disturb or reduce protein synthesis, which was often observed during ageing and cancer development [30].

In colitis, an inflammatory and preneoplastic state of CRC, MDA level was significantly elevated and antioxidants could ameliorate severity of colitis following a decrease of MDA concentration in animal models [31,32]. In different cancers, such as skin [33], breast cancer and also CRC [34], increased plasma or urine MDA concentration has been observed. In patients with advanced inoperable CRC, serum MDA concentration is much higher than in those with primary CRC [35]. Surineni et al. [36] also found that in 65 stage II/III CRC patients, serum MDA level was more elevated in stage III than stage II patients. The serum MDA level was significantly decreased after surgical treatments compared to presurgical status [36]. These observations suggest that MDA might relate to CRC carcinogenesis and progression. It might therefore be used as a biomarker for clinical prognosis and also be an index of surveillance of treatment effects.

The inhibitory effect of PUFAs in CRC cell growth is possibly due to an increase of reactive oxygen species and MDA level, which could consequently lead to mitochondrial dysfunction [24*]. The cytotoxic effect of MDA indicates its potential role in CRC treatment.
4-Hydroxy-2-nonenal: the most toxic product of lipid peroxidation

4-HNE is the most toxic product of peroxidation of PUFAs due to its high chemical reactivity and also its long half-life clearance. 4-HNE-induced protein modification was observed in different kinds of degenerative diseases and ageing [37,38]; 4-HNE mainly reacts with proteins, especially with tyrosine kinase-associated receptors; it can also induce severe DNA damage. These reactions lead to modification of cell cycle, cell signaling, inhibition/activation of related enzymes [39], which would consequently be carcinogenic.

Apart from its carcinogenic effect, high concentration of 4-HNE has an inhibitory effect on cell growth, whereas low concentration in vitro triggers cell proliferation [40]. Elevated 4-HNE is a potential mediator of mitochondrial permeability transition by reacting with calcium-ATPase, which can disrupt calcium homeostasis then induce a cascade reaction whose final outcome is cell death [37]. 4-HNE could also increase oxidative stress by promoting cellular consumption of glutathione, which is mediated by glutathione S-transferase and by inactivation of selenium-dependent glutathione peroxidase [41]. Mediated by the above mechanisms, 4-HNE modifies tumor suppressor protein activity that is associated with chronic inflammation [42]. Apart from proteins, 4-HNE could also react in vivo with DNA and cause DNA damage by producing 1,N_{6}{-}etheno-2'-deoxyadenosine, which is found to have a high urinary excretion in prehepatocellular carcinoma patients, compared to healthy volunteers [43]. These observations suggest that 4-HNE is involved in carcinogenesis in different cancers. The fact that more 4-HNE is found in invasive breast cancer compared with benign breast cancer [44] signifies its effect in cancer development.

In CRC, 4-HNE could significantly inhibit cancer cell growth in Caco-2 and HT-29 cell lines [45]. The effect is likely to be related with apoptosis induction and inhibition of telomerase activity. At the same time, a rapid decrease of glutathione, an intrinsic antioxidant, is observed after treatment of 4-HNE [45]. These observations imply that the anticancer effect of 4-HNE might be related to...
alteration of oxidative stress, which induces cancer cell apoptosis and inhibits telomerase activity. Furthermore, 4-HNE has different genotoxicity in different CRC cell lines, depending on their TP53 status [46]. Glutathione conjugates of 4-HNE are already used as chemotherapeutic agents in some cancer models [47]. Concomitant use of 4-HNE with radiotherapy in CRC has not yet been reported.

In conclusion, 4-HNE has a carcinogenic effect in normal cells, whereas its proapoptotic and suppressive effect in cancer cells is also observed. It has possibly a double-edged sword effect, which needs to be further investigated.

**Isoprostanes: in-vivo golden standard biomarker of lipid peroxidation**

Isoprostanes are so named because they contain F-type prostanate rings analogous to prostaglandin F2α (PGF2α). F2-isoprostanes, derived from ω-6 PUFAs, are the most studied species. ω-3 PUFAs mainly generate F3 and F4-isoprostanes [28].

F2-isoprostanes are considered as reliable biomarkers of lipid peroxidation in various inflammatory states, such as atherosclerosis, rheumatic diseases [48], chronic lung diseases [49], degenerative diseases, aging, obesity and cancers [50]. In obesity, one of the risk factors of CRC, F2-isoprostanes level is highly correlated with visceral and subcutaneous adipose tissue. High formation of 8-iso-PGF2α (main metabolite of F2-isoprostanes in vivo) is detected in women with BMI greater than 28 kg/m². Loss of weight has been linked to lower 8-iso-PGF2α level [48]. In an animal model of colitis, which is a precancerous lesion of CRC, a high urinary 8-iso-PGF2α level is detected [51]. The elevation of 8-iso-PGF2α existed in an animal model of CRC [52]. Thus, it appears that 8-iso-PGF2α has a...
correlation with cancer risk and also cancer development. In a randomized controlled clinical trial, long-term antioxidant micronutrient supplementation in patients with sporadic CRC was efficient in decreasing serum F₂-isoprostanes level, and also other biomarkers of inflammation, such as IL-6 and TNF-α [53]. As primary isoprostanes and their β-oxidized products are secreted in plasma and then efficiently eliminated in urine [48], F₂-isoprostanes and their metabolites are considered to be the most accurate biomarkers of oxidative stress for epidemiological studies [54]. Compared to F₂-isoprostanes, F₃ and F₄-isoprostanes are mainly derived from ω-3 PUFAs. A fish oil-enriched diet elevates dose-dependently the excretion of F₃ and F₄-isoprostanes in mice urine, following a decrease of F₂-isoprostanes [28]. F₂-isoprostanes but not F₁-isoprostanes could increase arterial pressure and irreversible platelet aggregation in mice [28]. The result indicates that F₂-isoprostanes have systemic proinflammatory effects in animal models, whereas F₁-isoprostanes have no such effect. And ω-3 PUFAs-derived F₂-isoprostanes competitively decrease the excretion of F₂-isoprostanes from ω-6 PUFAs [28]. Different production of F₃-isoprostanes rather than F₂-isoprostanes with fish oil-enriched diet might explain the benefits of ω-3 PUFAs in healthy animals. The direct effect of isoprostanes in CRC treatment has not yet been reported. The role of lipid peroxidation in cancer has been widely debated. Evidence demonstrates that lipid peroxidation plays a role in the carcinogenetic pathway in different human cancers, including CRC [55–57], because end products of lipid peroxidation are mutagenic and so may be probably involved in cancer development. However, MDA and 4-HNE also have cytotoxic effects in CRC cells. Therefore, combined treatment of ω-3 PUFA and radiotherapy or chemotherapy might emphasize CRC cell disruption and apoptosis by increasing lipid peroxidation [19]. An in-depth epidemiological and experimental evaluation of effects of ω-3 or ω-6 PUFA and their peroxidation end products on CRC radiotherapy and chemotherapy would thus be valuable and important for the development of new modalities for CRC treatment.

CONCLUSION

Accumulating evidence suggests that PUFAs are involved in both CRC development and treatment. The underlying mechanisms are yet not clearly demonstrated. Inflammation, induced by oxidative stress, may play a paramount role in cancer risk. The end products of lipid peroxidation, MDA, 4-HNE and isoprostanes, could be used as biomarkers for cancer detection, surveillance of treatment outcome and prognostic index for CRC patients. Furthermore, MDA and 4-HNE have cytotoxic effects not only in normal cells but also in CRC cancer cells, which implies the potential role of PUFAs and their end products of lipid peroxidation in CRC treatment.

Acknowledgements

None.

Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 201).

9. Hall MN, Chavarro JE, Lee IM, et al. A fish oil-enriched diet efficiently eliminated in urine [48]. F₂-isoprostanes and their metabolites are considered to be the most accurate biomarkers of oxidative stress for epidemiological studies [54].

18. This article illustrates the suppressive role of pure eicosapentaenoic acid in colorectal cancer development, which would be used as a preventive treatment.

This is a large, population-based prospective study, found that intake of marine ω-3 PUFAs may be inversely related to the risk of cancer in the proximal site of the large bowel. But there is no evidence that ω-6 PUFAs provoke colorectal cancer.


This article demonstrated that ω-6 PUFAs when used in a low concentration could promote cell proliferation. The cytotoxic effect was observed when ω-6 PUFAs was used in a higher concentration.

